

What is claimed is:

1. A method for detecting at least one target nucleic acid sequence in a sample comprising:

forming a ligation reaction composition comprising the sample and a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence,

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence, and wherein one probe in each probe set further comprises an addressable portion located between the primer-specific portion and the target-specific portion, wherein the addressable portion comprises a sequence;

forming a test composition by subjecting the ligation reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, the addressable portion, and the 3' primer-specific portion;

forming an amplification reaction composition comprising:

the test composition;

a polymerase;

a labeled probe, wherein the labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence, and wherein the labeled probe comprises the sequence of the

addressable portion or comprises a sequence complementary to the sequence of the addressable portion; and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

subjecting the amplification reaction composition to at least one amplification reaction; and

detecting a second detectable signal value at least one of during and after the amplification reaction, wherein a threshold difference between the first detectable signal value and the second detectable signal value indicates the presence of the target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second detectable signal value indicates the absence of the target nucleic acid sequence.

2. The method of claim 1, wherein the labeled probe is a 5' nuclease probe.

3. The method of claim 2, wherein the 5' nuclease probe comprises at least one signal moiety and at least one quencher moiety.

4. The method of claim 3, wherein the least one signal moiety comprises at least one fluorescent moiety.

5. The method of claim 2, wherein the 5' nuclease probe comprises at least one signal moiety and at least one donor moiety.
6. The method of claim 1, wherein the labeled probe is a hybridization dependent probe.
7. The method of claim 6, wherein the hybridization dependent probe comprises at least one signal moiety and at least one quencher moiety.
8. The method of claim 7, wherein the least one signal moiety comprises at least one fluorescent moiety.
9. The method of claim 6, wherein the hybridization dependent probe comprises at least one signal moiety and at least one donor moiety.
10. A method for detecting at least one target nucleic acid sequence in a sample comprising:
forming a ligation reaction composition comprising the sample and a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence,
wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid

sequence, and wherein one probe in each probe set further comprises an addressable portion located between the primer-specific portion and the target-specific portion, wherein the addressable portion comprises a sequence;

forming a test composition by subjecting the ligation reaction composition to at least one cycle of ligation, wherein adjacently hybridizing complementary probes are ligated to one another to form a ligation product comprising the 5' primer-specific portion, the target-specific portions, the addressable portion, and the 3' primer-specific portion;

forming an amplification reaction composition comprising:

the test composition;

a polymerase;

a labeled probe, wherein the labeled probe comprises the sequence of the addressable portion or comprises a sequence complementary to the sequence of the addressable portion; and

at least one primer set, the primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-specific portion of the ligation product, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the ligation product;

subjecting the amplification reaction composition to at least one amplification reaction; and

detecting the presence or absence of the target nucleic acid sequence by monitoring a signal at least one of during and after the at least one cycle of amplification.

11. A kit for detecting at least one target nucleic acid sequence in a sample comprising:

a ligation probe set for each target nucleic acid sequence, the probe set comprising (a) at least one first probe, comprising a target-specific portion and a 5' primer-specific portion, wherein the 5' primer-specific portion comprises a sequence, and (b) at least one second probe, comprising a target-specific portion and a 3' primer-specific portion, wherein the 3' primer-specific portion comprises a sequence,

wherein the probes in each set are suitable for ligation together when hybridized adjacent to one another on a complementary target nucleic acid sequence, and wherein one probe in each probe set further comprises an addressable portion located between the primer-specific portion and the target-specific portion, wherein the addressable portion comprises a sequence; and

a labeled probe comprising the sequence of the addressable portion or comprising a sequence complementary to the sequence of the addressable portion.

12. The kit of claim 6, wherein the labeled probe has a first detectable signal value when it is not hybridized to a complementary sequence and a second detectable signal value of the labeled probe can be detected at least one of during and after an amplification reaction, wherein a threshold difference between the first detectable signal value and the second detectable signal value indicates the presence of the target nucleic acid sequence, and wherein no threshold difference between the first detectable signal value and the second detectable signal value indicates the absence of the target nucleic acid sequence.

13. The kit of claim 11, wherein the labeled probe is a 5' nuclease probe.
14. The kit of claim 13, wherein the 5' nuclease probe comprises at least one signal moiety and at least one quencher moiety.
15. The kit of claim 14, wherein the least one signal moiety comprises at least one fluorescent moiety.
16. The kit of claim 13, wherein the 5' nuclease probe comprises at least one signal moiety and at least one donor moiety.
17. The kit of claim 11, wherein the labeled probe is a hybridization dependent probe.
18. The kit of claim 17, wherein the hybridization dependent probe comprises at least one signal moiety and at least one quencher moiety.
19. The kit of claim 18, wherein the least one signal moiety comprises at least one fluorescent moiety.
20. The kit of claim 17, wherein the hybridization dependent probe comprises at least one signal moiety and at least one donor moiety.
21. The kit of claim 11, further comprising at least one primer set comprising (i) at least one first primer comprising the sequence of the 5' primer-

specific portion of the at least one first probe, and (ii) at least one second primer comprising a sequence complementary to the sequence of the 3' primer-specific portion of the at least one second probe.